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Review

Gastrin-releasing peptide and cancer

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Abstract

Over the past 20 years, abundant evidenee has been eolleeted to suggest that gastrin-releasing peptide (GRP) and its receptors play an important role in the development of a variety of eaneers. In faet, the deteetion of GRP and the GRP receptor in small cell lung carcinoma (SCLC), and the demonstration that anti-GRP antibodies inhibited proliferation in SCLC eell lines, established GRP as the prototypical autocrine growth factor. All forms of GRP are generated by proeessing of a 125-amino aeid prohormone; reeent studies indieate that C-terminal amidation of GRP_{18-27} is not essential for bioactivity, and that peptides derived from residues 31 to 125 of the prohormone are present in normal tissue and in tumors. GRP receptors can be divided into four elasses, all of whieh belong to the 7 transmembrane domain family and bind GRP and/or GRP analogues with affinities in the nM range. Over-expression of GRP and its reeeptors has been demonstrated at both the mRNA and protein level in many types of tumors including lung, prostate, breast, stomaeh, panereas and eolon. GRP has also been shown to act as a potent mitogen for cancer cells of diverse origin both in vitro and in animal models of eareinogenesis. Other aetions of GRP relevant to carcinogenesis include effects on morphogenesis, angiogenesis, cell migration and eell adhesion. Future prospeets for the use of radiolabelled and eytotoxic GRP analogues and antagonists for cancer diagnosis and therapy appear promising.

Keywords: Bombesin; GRP; Migration; Proliferation

Contents

1. Introduction . . . 24
2. Gastrin-releasing peptide (GRP) . . . 24
   2.1. Discovery of GRP and GRP mRNA . . . 24
   2.2. Processing of preproGRP . . . 25
   2.3. Detection and expression of GRP in normal tissue . . . 26
3. Receptors for the GRP Family . . . 26
   3.1. Molecular biology of GRP receptors . . . 26
   3.2. Binding properties of GRP reeeptors . . . 26
   3.3. Loealization of GRP reeeptors in normal tissue . . . 27
      3.3.1. GRP-R . . . 27
      3.3.2. NMB-R . . . 27
      3.3.3. BRS-3 . . . 27
4. Effects in normal tissue . . . 27
   4.1. Mitogenic effeets . . . 28
   4.2. Protective effects . . . 28

Abbreviations: GRP, gastrin-releasing peptide; GRPgly, glycine-extended GRP

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5. Detection of GRP and GRP receptors in tumors . . . 28
5.1. GRP . . . 28
5.1.1. Lung cancer . . . 28
5.1.2. Prostate cancer . . . 29
5.1.3. Breast cancer . . . 29
5.1.4. Gastric cancer . . . 29
5.1.5. Pancreatic cancer . . . 29
5.1.6. Colorectal cancer . . . 29
5.1.7. Gastrointestinal carcinoid tumors . . . 29
5.2. GRP-R and related receptors . . . 29
5.2.1. Lung cancer . . . 29
5.2.2. Prostate cancer . . . 30
5.2.3. Breast cancer . . . 30
5.2.4. Gastric cancer . . . 30
5.2.5. Pancreatic cancer . . . 30
5.2.6. Colorectal cancer . . . 30
5.2.7. Gastrointestinal carcinoid tumors . . . 31
6. Mitogenic role of GRP in cancer . . . 31
6.1. Lung cancer . . . 31
6.2. Prostate cancer . . . 31
6.3. Breast cancer . . . 32
6.4. Gastric cancer . . . 32
6.5. Pancreatic cancer . . . 32
6.6. Colorectal cancer . . . 32
7. Morphogenic role of GRP . . . 33
8. Biological activity of non-amidated GRPs . . . 33
9. Prospects for therapy . . . 34
10. Summary and future directions . . . 34
References . . . 34

1. Introduction

The autocrine hypothesis for cancer growth postulates that a cell produces both a growth factor and its cognate receptor, and that their interaction results in proliferation. The recognition of gastrin-releasing peptide (GRP) as the prototypical autocrine growth factor was originally based on the detection of GRP and its cognate receptor, and on the anti-proliferative effect of GRP antibodies, in small cell lung carcinoma (SCLC) [1]. However, GRP is also a potent mitogen for several other types of carcinomas including colon, pancreas, prostate and breast [2], and the signalling pathways involved in mitogenesis have been studied extensively [3]. Recent data show that GRP is not only an autocrine mitogen, but also has paracrine and endocrine effects, and functions as a morphogen and a proangiogenic agent. The presence of high affinity GRP receptors in many cancers [3] has also allowed the development of reagents for diagnosis, radiotherapy and chemotherapy [4].

In this review, current knowledge of the role of GRP in cancer will be summarised. We will begin by covering the discovery of the amphibian peptide bombesin and its mammalian relative GRP, and outlining the advances in molecular biology that led to the elucidation of the structures of the peptides and their receptors. We will then summarise the distribution of GRP and its receptors in normal tissue and in various tumors, and consider the evidence for their involvement in carcinogenesis, and the use of GRP analogues for diagnosis and therapy. We will emphasize the developments over the last decade; earlier studies have been reviewed previously [2].

2. Gastrin-releasing peptide (GRP)

2.1. Discovery of GRP and GRP mRNA

The tetradecapeptide bombesin was first isolated and characterized from the skin of the amphibian *Bombina bombina* [5]. A decade elapsed before researchers, using antisera developed against bombesin, identified the mammalian counterpart of bombesin, which was named gastrin-releasing peptide (GRP) after its first known activity of inducing gastrin secretion from G cells in the gastric antrum [6]. Shortly thereafter a novel bombesin-like peptide was identified in porcine spinal cord and named neuromedin B (NMB) [7]. All three peptides are related in sequence and share an amidated methionine at their carboxyl termini (C-termini) (Fig. 1). Like many neuropeptides mature amidated GRP arises by a series of well-defined processing steps from an initial large translation product (preproGRP).

The cDNA encoding human GRP was first cloned from the hepatic metastasis of a pulmonary carcinoid tumor [8]. Further analysis revealed that there are three GRP mRNAs in humans, which all identically encode GRP_{1-27} and GRP_{18-27}, but differ in the sequence encoding the C-terminal extension peptide [9,10]. The three GRP mRNAs arise from a single nascent mRNA by alternate splicing at the junction between exons 2 and

GRP

Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Bombesin

pGlu-Gln-Arg-Leu-**Gly-Asn**-Gln-**Trp-Ala-Val-Gly-His-Leu-Met-NH_2**

NMB

Gly-Asn-Leu-**Trp-Ala**-Thr-**Gly-His**-Phe-**Met-NH_2**

Fig. 1. Sequences of bombesin and GRP. The C-termini of bombesin, first isolated from the skin of the frog *Bombina bombina* [5], human gastrin-releasing peptide (GRP) [6] and porcine neuromedin B (NMB) [7], are very similar, with a common C-terminal methionine amide. Amino acids are shown in the standard three letter code, and identical residues are shown in bold.

3, and encode peptides of 148, 141, and 138 amino acids. The functional differences, if any, between the four forms have not been established, but interestingly, the fourth possible splice variant was not detected in humans.

2.2. Processing of preproGRP

Processing of preproGRP in mammalian tissues and in cultured cells takes place at discrete sites (Fig. 2). Initially, the first 23 amino acids of preproGRP are removed by a signal peptidase, and proGRP fragments are therefore numbered from the amino terminal valine. The endopeptidase that processes proGRP after the double lysine at positions 29 and 30 has not been identified, but it has been postulated that the processing is mediated by prohormone convertase 2 [11]. Carboxypeptidase B-like activity removes the two basic residues from the C-terminus to form glycine-extended $GRPgly_{1-28}$. C-terminal amidation is then catalyzed by the sequential action of peptidylglycine alpha-hydroxylating monooxygenase (PHM) and peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL), which together are known as peptidylglycine alpha-amidating monooxygenase (PAM) [12]. PAM removes glyoxylate from glycine28 and leaves amidated methionine27 at the C-terminus. The mature form of human GRP contains 27 amino acids, but an additional cleavage that yields GRP_{18-27} may occur between arginine17 and glycine18 [13]. It is not known whether this cleavage takes place before or after amidation. Multiple cleavages of the C-terminal extension

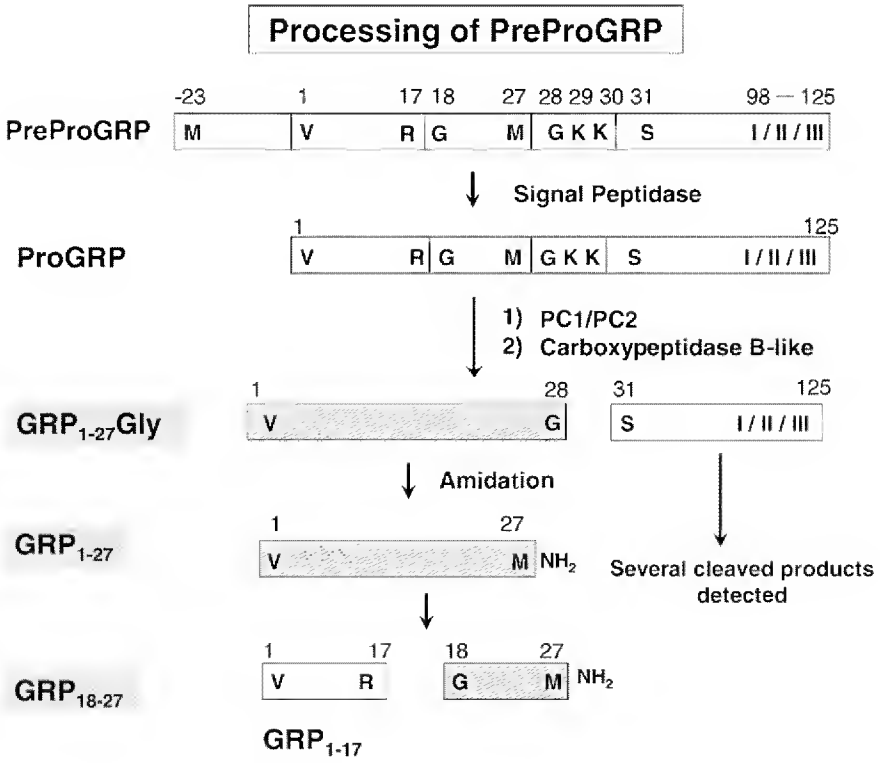

Fig. 2. Processing of preproGRP. The processing of proGRP is initiated by a signal peptidase that removes the signal peptide from all three forms of preproGRP (Forms I, II and III). The endopeptidase that processes proGRP after the double lysine (Lys–Lys) at positions 29 and 30 has not been identified, but it is postulated that the processing is mediated by prohormone convertase 2 [11]. A carboxypeptidase B-like activity then removes basic residues from the new C-terminus to form the glycine-extended form of GRP (GRPgly). The amidation enzyme peptidyl glycine α-amidating monooxygenase (PAM) finally removes glyoxylate from glycine28 and leaves methionine27 with a C-terminal amide. A trypsin-like activity may cleave between amino acids 17 and 18 giving rise to amidated GRP_{18-27}. The relative contribution of the alternative processing pathway in which cleavage between amino acids 17 and 18 occurs prior to amidation is not known. The molecules known to be biologically active (i.e. GRP_{1-27}, GRP_{18-27} and GRP_{18-27}gly) are shaded.

peptide ($proGRP_{31-125}$) also occur in a tissue specific manner [14,15].

2.3. Detection and expression of GRP in normal tissue

The discovery of GRP, and the availability of antiserum against this peptide, led to the demonstration of immunoreactivity in tissue extracts and the immunocytochemical localization of GRP. Cloning of the cDNA encoding GRP made possible the use of Northern analysis and in situ hybridization to investigate the expression of GRP mRNA in different tissues and cell types. The availability of the GRP nucleotide sequence also allowed the design of primers which permitted investigators to use RT-PCR to detect the GRP mRNA in cells and tissues.

The regional distribution of GRP within the brain and central nervous system has been described by a number of groups [16,17]. Using an antiserum which recognizes the C-terminus of bombesin, Moody and co-workers detected low densities of bombesin-like peptides in the cortex, whereas high densities were present in the hindbrain, midbrain and hypothalamus [16]. GRP is also found in the spinal cord especially in sensory ganglia [17].

GRP is present in nerve fibres throughout the gastrointestinal tract and pancreas [18]. Both the oxyntic and antral mucosa are innervated by GRP with influences on acid secretion and gastrin release respectively [19], although the effect on gastrin release has been questioned recently [20]. In the most comprehensive study of the large and small intestine, Costa and colleagues reported that GRP immunoreactive nerves were present in the myenteric plexus, circular muscle, submucous plexus and in the celiac ganglion of guinea pigs [21].

The occurrence of GRP immunoreactivity in human lung has been well documented in both adult [22,23] and fetal tissue [24]. In specimens of lung collected from fetuses of gestational aged 15–22 weeks, no correlation could be established between gestational age and bombesin-like immunoreactivity [25] Yamaguchi and co-workers using a radioimmunoassay specific for the C-terminus of GRP detected immunoreactive GRP in all (5/5) fetal lung samples [23]. In fetal lungs, GRP is stored in a single cell type, the P1 endocrine cell of the airway epithelium [26].

GRP has also been detected in other human tissues. The pregnant uterus of a number of species including the human and sheep is a major site of GRP production although most of the GRP appears to be incompletely processed [27,28]. Other sources of GRP include the pancreas [29], thymus [30], prostate and urethra [31].

3. Receptors for the GRP Family

Four different receptor subtypes (GRP-R, NMB-R, BRS-3 and BB4-R) have been described for the GRP family of peptides. All the GRP receptors characterized to date are guanine nucleotide binding protein (G-protein)-coupled, have seven transmembrane domains, and activate phospholipase C to increase intracellular concentrations of inositol phosphates, diacyl glycerol and calcium [32]. Other intracellular mediators activated by GRP receptors include mitogen-activated protein kinase, focal adhesion kinase, phosphatidylinositol 3-kinase and in some situations cyclic AMP response element-binding protein [33–35].

An alternative nomenclature system for the GRP receptors is also in use. In this system the NMB receptor, GRP receptor and BRS-3 are known as the bombesin1 receptor (BB1-R), the BB2 receptor (BB2-R) and the BB3 receptor (BB3-R), respectively, and the fourth receptor is known as the BB4 receptor (BB4-R).

3.1. Molecular biology of GRP receptors

In 1990, two independent research groups isolated and characterized cDNA clones encoding the mouse GRP-R from Swiss 3T3 cells [36,37]. The human GRP-R was then cloned by hybridization at low stringency, using the mouse receptor cDNA as probe [38]. Sequence and hydrophobicity analysis revealed the GRP-R to be 384 amino acids long and a member of the seven transmembrane domain receptor family.

Binding studies in rat brains and other tissues provided evidence that GRP and NMB exerted their physiological effects through at least two different receptors [39]. The NMB-R cDNA was then cloned from rat oesophagus [40] and from human genomic libraries [41] using a GRP-R cDNA probe under low stringency hybridization conditions. Further analysis revealed that the receptor is composed of 390 amino acids and belongs to the seven transmembrane domain superfamily.

Two other subtypes of the GRP receptor family have been described. The third subtype designated BRS-3 was cloned from guinea pig uterus and from a human placental cDNA library [42,43]. A putative fourth member of the GRP receptor family (BB4-R) was isolated from a cDNA library from the frog *Bombina orientalis* [44]. The cloned BRS-3 and BB4-R cDNAs encode proteins of 399 and 392 amino acids, respectively, both of which are members of the seven transmembrane domain receptor superfamily. A mammalian counterpart of the frog BB4-R receptor has not yet been identified.

3.2. Binding properties of GRP receptors

Receptors of the GRP-R family can be readily distinguished on the basis of agonist and antagonist affinities (Table 1). The GRP-R has high affinity for bombesin and GRP and very low affinity for NMB. The NMB-R had a higher affinity for NMB than GRP when expressed in BALB 3T3 fibroblasts [40]. Radioligand displacement assays with the transfected BRS-3 cDNA revealed an extremely low affinity for GRP, bombesin and NMB. Rational design of a BRS-3 agonist has given rise to a universal ligand which binds to all four receptor subtypes with high affinity [45]. Although no known human tissues or cell lines possess sufficient numbers of BB4-R receptors to allow their cell biology to be determined, the development of stable clones of CHO-K1 cells expressing amphibian BB4-R has permitted the determination of its unique pharmacology [46]. The failure to observe a BB4-R homologue in primates suggests

Table 1
Binding properties of receptors of the GRP-R family

Peptide	Receptor Affinity (nM)			
	GRP-R	NMB-R	BRS-3	BB4-R
Bombesin	2.1±0.1[a]	32±2[g]	>10000[e]	14±1[f]
GRP	6.6±1.3[a]	4800[d]	>10000[e]	79±12[f]
NMB	700±200[a]	1.8±0.3[e]	>10000[e]	11±1[f]
BRS-3 agonist	0.2±0.07[e]	9±0.8[e]	0.32±0.8[e]	0.41±0.06[f]
GRP-R antagonist [D-Phe[6], Leu-NHet[13], des-Met[14]]-bombesin(6–14)	16[b] to 27[c]	>10000	ND	62±11[f]

Cell lines and references are as follows: [a]human CRC cell line NCI-HCT716 [243], [b]guinea pig pancreatic acini [244], [c]mouse Swiss 3T3 fibroblasts [245], [d]mouse Balb/C 3T3 fibroblasts stably transfected with the human NMB-R [246], [e]same cell line as [d] [247], [f]Chinese hamster ovary CHO-K1 cells stably transfected with the frog BB4-R [46], [g]same cell line as [d] [248].

that the expression of BB4-R may be limited to amphibians [47].

3.3. Localization of GRP receptors in normal tissue

The following three sections summarise some of the studies which have demonstrated expression of receptors of the GRP-R family in various fetal or adult tissues. Different methods have been used but the most common detected mRNA expression using RT-PCR.

3.3.1. GRP-R

GRP-R mRNA has been detected by in situ hybridization in the mesenchyme around developing airways and blood vessels in mice, in epithelial cells in rodents, humans and non-human primates, and in human endothelial cells [40,48–52]. Although GRP-R expression was reported in peripheral blood cells from healthy human subjects using RT-PCR [53,54], Bessho and co-workers detected no GRP-R mRNA in blood and bone marrow from 20 healthy volunteers [55]. Ferris and colleagues performed RT-PCR on extracted RNA obtained from endoscopic pinch biopsies of epithelial cells lining the esophagus, stomach, jejunum, ileum, and proximal and descending colon in the human gastrointestinal tract. GRP-R expression was limited to cells lining the gastric antrum, indicating that receptor expression in gastric cancers is aberrant [56]. GRP-R mRNA is also abundant in human pancreas where four transcripts of different sizes were observed [57]. Dumesny and co-workers have shown that GRP-R mRNA is expressed in the human embryonic kidney cell line HEK293 and in fetal and adult sheep and rat kidneys [58]. Gugger and Reubi demonstrated moderate- to very high-density expression of the GRP family of receptors including GRP-R in both lobules and ductules of human non-neoplastic breast glands, using radiolabelled bombesin or the universal ligand D-Tyr[6], β-Ala[11], Phe[13], Nle[14]-bombesin(6–14) [59]. GRP-R but not NMB-R or BRS-3 binding sites were also detected in the myometrium and endometrium of normal human uterus [60].

GRP-R may also be expressed transiently during fetal development. In situ hybridization studies in rats have shown that GRP-R mRNA is expressed at early embryonic stages at various locations in the nervous, urogenital, respiratory, and gastrointestinal systems [61]. Transient GRP-R mRNA expression was observed in rat posterior pituitary where expression increased from embryonic day 12 to day 20, and abruptly disappeared at birth [61]. Similarly, co-expression of GRP and GRP-R protein was observed in enterocytes of rat intestinal villi between days N-1 and N-12, and of human intestinal villi between weeks 14 and 20 post-conception [62].

3.3.2. NMB-R

Initial studies suggested that the distribution of NMB-R mRNA expression is restricted to the upper gastrointestinal tract, bladder, and central nervous system and is observed at later embryonic stages [61,63]. In most locations, receptor mRNA increased steadily throughout development [61]. Chave and co-workers failed to detect NMB-R mRNA expression in human colon cancers or in matched normal tissue [63]. In contrast to the result of Chave et al., Matusiak and co-workers detected NMB-R expression in normal colon by RT-PCR and immunohistochemistry [64]. Expression of NMB-R genes was also detected in the mouse lung from embryonic days 13–16 and on multiple postnatal days, and expression was down-regulated by bombesin treatment [65]. In rat brain, expression of NMB-R mRNA, but not GRP-R or BRS-3 mRNAs, was observed in olfactory regions and in the thalamus. In many other brain areas, including isocortex, hippocampus, amygdala, hypothalamus, and brain stem, NMB-R was co-localized with GRP-R [66]. Vigne and co-workers have shown that endothelial cells isolated from rat brain microvessels express a NMB-preferring receptor involved in the control of potassium ion secretion across the blood/brain barrier [67]. Human endothelial cells have also been shown by PCR to express abundant NMB-R mRNA [52].

3.3.3. BRS-3

Expression of BRS-3 mRNA has been detected in primate fetal lung [48] and in human bronchial epithelial cells [68], in the uterus of the pregnant guinea pig [43] and in rat testis [42]. Within the CNS, BRS-3 mRNA expression has been observed in guinea pig [43] and mouse brain [69] but was not detected in rat brain by Northern analysis [42]. In the ewe, BRS-3 mRNA expression was detected in pituitary and hypothalamus but not in pregnant uterus (endometrium, myometrium, chorioallantois or amnion) or in non-pregnant uterus or testis [70]. Expression of the BRS-3 gene was detected in mouse lung from embryonic days 13 to 16 and on multiple postnatal days, and expression was not affected by bombesin treatment [65]. Expression of BRS-3 mRNA was also detected in the fetal and adult rat kidney [58]. In contrast to the high expression of NMB-R mRNA, no BRS-3 mRNA was detected in human endothelial cells [52].

4. Effects in normal tissue

The GRP-like peptides play many physiological roles in addition to the stimulation of gastric acid secretion [18]. As well as stimulating the release of a variety of hormones including

gastrin, somatostatin and CCK, the GRP-like peptides promote exocrine secretion from the pancreas, and smooth muscle contraction in stomach, small intestine, and several other tissues [18]. The neural effects of GRP-like peptides include changes in animal behaviour, suppression of food intake, and improvement of learning and memory in rodent models [71–74]. In this review, which focuses on GRP and cancer, we will consider only the abilities of GRP-like peptides to stimulate cell proliferation and to protect the gastrointestinal mucosa against noxious chemicals.

4.1. Mitogenic effects

Bombesin and GRP stimulate growth of the gastrointestinal mucosa and pancreas. In neonatal rats bombesin given orally or subcutaneously stimulated growth of the gastrointestinal mucosa and pancreas, as demonstrated by increases in gastric weight, in fundic and antral mucosal height, and in the density of parietal cells [75,76]. Dembinski and co-workers demonstrated that bombesin treatment caused a significant increase in the weight and RNA and DNA contents of the oxyntic mucosa of the stomach and the duodenal mucosa. They also suggested that the growth-promoting effects of bombesin/GRP involved the release of gastrin and cholecystokinin (CCK) and were mediated by the GRP-R receptor [77,78]. Bombesin prevented jejunal mucosal atrophy when rats were administered liquid elemental diets, and bombesin also significantly increased ileal mucosal growth compared to control [79]. Chu and co-workers demonstrated that bombesin increased mucosal weight, DNA and protein content in both jejunal and ileal Thiry-Vella fistulas in rats [80]. In a lethal methotrexate-induced enterocolitis model in rats, bombesin enhanced gut mucosal growth and significantly decreased mortality [81]. Bombesin has also been shown to increase the labelling index of epithelial cells of rat colonic mucosa [82,83], and GRP-R-deficient mice displayed complete attenuation of colonic villous growth between days N1 and N12 [62]. A number of studies have shown that bombesin/GRP stimulated growth of pancreas [84,85], and chronic bombesin administration has been shown to stimulate pancreatic regeneration after pancreatectomy in pigs [86].

4.2. Protective effects

Bombesin protects the mucosa against damage in several animal models of gastrointestinal injury. Exogenous bombesin prevents gastric injury induced by ethanol [87] and significantly improves the healing of the gastric mucosa of rats after acetic acid-induced ulcer induction [88]. Colonic damage induced by trinitrobenzene sulfonic acid in rats [89], and burn-induced gut malfunction were also ameliorated by bombesin treatment [90]. Bombesin-induced gastroprotection involves release of endogenous gastrin [87].

There have been isolated reports of additional protective functions of GRP. Bombesin improved intestinal barrier function and oxidative stress in rats with experimentally induced jaundice [91] or following partial hepatectomy [92].

5. Detection of GRP and GRP receptors in tumors

Apart from its physiologieal roles, GRP has been shown to be mitogenic for a number of cancerous tissues. The field was initiated by the demonstration by Cuttitta and co-workers that a monoclonal antibody that bound to the C-terminal region of GRP blocked the binding of the peptide to cellular receptors and inhibited the growth of SCLC cells in vitro and of SCLC xenografts in vivo [1]. The conclusion that GRP may be an autocrine growth factor in SCLC prompted various research groups to investigate the role of GRP in cancer development and progression. Tumors arising from various tissues including prostate, breast, stomach and colon were shown by RT-PCR, radioimmunoassay and/or radioligand binding assays to express either GRP or GRP-R or both, as discussed in the following sections and summarised in Table 2. It should be remembered that detection of GRP or GRP-R mRNA by PCR is not necessarily indicative of peptide or receptor protein expression, respectively.

5.1. GRP

5.1.1. Lung cancer

Human SCLC cells have been shown to contain significant concentrations of GRP. Using RT-PCR amplification, GRP mRNA was detected in several SCLC cell lines (LU165, SBC1, SBC2, and SBC3) but not in non-SCLC cell lines (A549, ABC1, EBC1, and Oka-1). GRP-like immunoreactivity has also

Table 2
Expression of GRP and its receptor in tumors

Tumor	GRP				GRP-R			
	No. positive/ Total no.	%	Method	Ref.	No. positive/ Total no.	%	Method	Ref.
Lung (SCLC)	23/31	74	RIA	[98]	17/20	85	PCR	[115]
	NS	71	RIA	[249]	11/38	29	PCR	[53]
	9/20	45	RIA	[250]	3/9	33	Binding	[116]
	16/32	50	PCR	[97]				
Prostate	18/30	60	IH	[102]	30/30	100	Binding	[119]
	27/41	64	IH	[251]	50/80	63	Binding	[120]
					20/22	91	PCR	[120]
					12/12	100	Binding	[116]
Breast	16/41	39	RIA	[104]	33/100	33	Binding	[124]
	5/28	18	NB	[105]	29/46	63	Binding	[59]
					41/57	72	Binding	[116]
Gastric					13/23	50	Binding	[125]
					8/20	40	PCR	[126]
Pancreatic	52/88	59	RIA	[106]	2/26	8	PCR	[132]
					2/12	17	Binding	[133]
Colorectal	42/50	84	IH	[109]	6/15	40	Binding	[144]
	23/23	100	PCR	[63]	5/21	24	Binding	[147]
					27/29	93	PCR	[148]
					38/50	76	IH	[109]
					23/23	100	PCR	[63]
Carcinoids	12/20	60	IH	[113]	22/26	85	IH	[114]
	9/19	47	IH	[114]				

The abbreviations are: IH, immunohistochemistry; NB, Northern blot; NS, not stated; PCR, polymerase chain reaction; RIA, radioimmunoassay.

been described in SCLC and carcinoid cell lines, but not in non-SCLC cell lines [93,94]. Similar patterns of GRP expression, consisting mainly of GRP18–27 and some GRP14–27, together with large fragments of C-terminal proGRP, were observed by gel filtration chromatography in extracts of three SCLC cell lines (NIC-H345, NIC-H69, and NIC-H510) [95,96].

In patients with SCLC, GRP mRNA was detected in 50% (16/32) of blood samples, 18% (2/11) of marrow samples, and all pleural effusion samples (6/6) [97]. Blood samples from 58% (11/19) of the patients with extensive disease gave positive results as compared to 38% (5/13) of patients with limited disease. In contrast, less than 3% (1/38) of blood samples from patients with non-SCLC lung adenocarcinoma gave a positive result [97]. Immunoreactive GRP was found in 74% of human SCLC examined (23/31), and 29% (9/31) of these contained large amounts of immunoreactive GRP (0.1 to 13 nmol/g) [98]. In the case of lung carcinoid tumors, immunoreactive GRP was found in 42% (5/12) of the cases examined, and large amounts of immunoreactive GRP were detected in two cases (5 to 120 nmol/g) [23]. Using an assay that measures proGRP31–98 (part of the C terminal flanking peptide of GRP) a number of groups have reported elevated circulating concentrations of proGRP [99,100]. The observation that proGRP concentrations were increased in more than 80% of patients with SCLC and decreased following resection suggests that it may be a useful marker for diagnosis, treatment and monitoring [99,100]. Indeed, in one report, it was the only significant prognostic marker [101]. Interestingly as noted above, SCLC cell lines also produce large amounts of C-terminal flanking peptides [95,96].

5.1.2. Prostate cancer

GRP expression was evaluated by immunohistochemistry in both biopsy and radical prostatectomy specimens from 30 patients with prostatic adenocarcinomas. GRP was expressed in 60% (18/30) of the radical prostatectomy specimens and in 50% (15/30) of the biopsies [102]. Both prostatic carcinomas and benign prostatic hyperplasia specimens stained for GRP [103]. Circulating proGRP31–98 was elevated in some patients with prostatic cancer although there was some overlap with patients with benign prostatic hyperplasia [103].

5.1.3. Breast cancer

In human breast carcinoma biopsies from post-menopausal patients, GRP immunoreactivity was detected in 39% (16/41) of the samples [104]. GRP mRNA was detected in 18% (5/28) of primary breast carcinomas but not in 6 mammary cancer cell lines [105].

5.1.4. Gastric cancer

There have been no reports on the expression of GRP in gastric adenocarcinomas.

5.1.5. Pancreatic cancer

GRP immunoreactivity was detected in 59% (52/88) of pancreatic ductal adenocarcinomas [106]. The presence of GRP mRNA in both well-differentiated (HPAF, CD11) and poorly differentiated (CD18, PANC-1) human ductal pancreatic cancer cell lines was demonstrated by primer extension analysis [107]. All four cell lines also secreted GRP immunoreactivity [107], and production of autocrine GRP by the HPAF cell line increased three-fold after weaning from its dependence on fetal bovine serum [108]. The proliferation of weaned cells cultured continuously in the same medium was significantly greater than of non-weaned cells [108].

5.1.6. Colorectal cancer

Few studies have investigated the presence of GRP in colon cancer. 84% of 50 randomly selected resected colon cancers expressed GRP or GRP-R as detected by immunohistochemistry, with 62% expressing both ligand and receptors. In contrast, the observation that less than 3% (1/37) of metastases expressed GRP or GRP-R [109] indicated that ligand and receptor expression in metastases and in the primary tumor do not necessarily correlate. Chave and co-workers detected GRP mRNA using RT-PCR in all of 23 colorectal cancer samples but not in matched normal mucosa [63]. In contrast, NMB mRNA was present in normal mucosa, but expression was generally greater in the tumor samples [63,64]. Increased GRP expression as detected by immunocytochemistry correlated with poor histological grade of colorectal adenocarcinomas [110]. Although GRP has been detected in colon cancer by immunohistochemistry, no studies have quantified GRP peptide concentrations by radioimmunoassay, or tested for larger, non-amidated forms of GRP by chromatography.

In an extensive study, GRP mRNA was detected in nine colon cancer cells lines, with concentrations ranging from 1100 to 6500 cDNA copies/μg tRNA [111]. In a subsequent study two of the cell lines (HT-29, Caco-2) also expressed NMB mRNA [64]. An independent study of HT-29 cells detected NMB, but not GRP, mRNA by RT-PCR [112].

5.1.7. Gastrointestinal carcinoid tumors

Gastrointestinal carcinoid tumors are low grade, malignant epithelial neoplasms showing neuroendocrine differentiation. Early reports indicated that GRP was frequently expressed in carcinoids in the small intestine (7/10, 70%) and appendix (4/5, 80%), but rarely in colonic carcinoids (1/5, 20%) [113]. Investigation of the expression of GRP and GRP-R by immunohistochemistry in a panel of 26 carcinoid tumors from the stomach, small intestine, appendix and colorectum revealed GRP in 47% (9/19) cases. Interestingly, no GRP staining was detected in the tumors originating from colon [114].

5.2. GRP-R and related receptors

5.2.1. Lung cancer

The GRP-R subtype was shown by RT-PCR to be the most commonly expressed GRP receptor in patients with lung cancer, being detected in 85% (17/20) of SCLC and 85% (11/13) of non-SCLC cases [115]. About a third of SCLC expressed the BRS-3 receptor measured by either RT-PCR or by in vitro receptor autoradiography [115, 116]. GRP-R expression was detected in 10% (4/40) of bone marrow aspirates and 29% (11/38) of peripheral blood samples [53]. GRP-R mRNA was

detected in two SCLC cell lines (NCI-H69 and NCI-H345) by RNase protection assay and by RT-PCR [117]. Expression of BRS-3 mRNA has also been detected in human lung carcinoma cells [42].

5.2.2. Prostate cancer

Using RT-PCR and in situ hybridization, widespread but variable GRP-R mRNA expression was demonstrated in fresh frozen specimens of prostate carcinoma (12 cases) and benign prostate hypertrophy (6 cases). GRP-R mRNA concentrations in cancerous tissue ranged widely from very high to undetectable (about 30% of cases), while normal tissue consistently displayed a low GRP-R mRNA concentration [118]. Using ^{125}I-Tyr^4-bombesin as a radioligand, GRP-R was detected in 100% of invasive prostate carcinomas (30/30) and intraepithelial prostate neoplasms (26/26). GRP-R were identified in only a few hyperplastic prostate glands, where they localized at a very low density in glandular tissue and focally in some stromal tissue [119]. Of the 80 specimens of primary prostate cancer examined by receptor binding assays with ^{125}I-Tyr^4-bombesin, 50 (63%) showed high-affinity, low-capacity binding sites for bombesin/GRP, and 12 of these 50 receptor-positive specimens possessed two classes of binding sites [120]. In the same study, 91% (20/22) of prostate cancer specimens analysed by RT-PCR expressed GRP-R mRNA [120]. GRP-R binding sites were detected in 12/12 prostate carcinomas but these same samples were negative for BRS-3 [116]. GRP receptors have also been demonstrated on several prostate carcinoma cell lines [121,122].

5.2.3. Breast cancer

The majority of breast carcinomas express GRP receptors and these are absent from normal breast epithelial cells [116]. GRP receptors were found in 38% (3/8) of breast cancer cell lines but not in 2 long-term cultures of normal human breast epithelial cells [123]. Halmos and co-workers investigated the binding of [^{125}I]-Tyr^4-bombesin to membranes isolated from 100 human breast carcinomas, and found that 33% expressed GRP receptors [124]. Gugger and co-workers evaluated GRP receptor expression in human non-neoplastic and neoplastic breast tissues using in vitro receptor autoradiography [59]. GRP receptors were detected, often in high density but with heterogeneous distribution, in neoplastic mammary epithelial cells in 63% (29/46) of invasive ductal carcinomas and in 65% (11/17) of ductal carcinomas in situ [59]. A comprehensive study by in vitro receptor autoradiography of the expression of GRP receptor subtypes found that 72% (41/57) of breast cancers expressed GRP receptors [116]. BRS-3 and NMB-R binding sites were absent from all 57 samples.

5.2.4. Gastric cancer

About half (13/23) of freshly resected gastric cancers expressed the GRP receptor, which was detected in only 1/23 samples of normal gastric mucosa [125]. Carroll and co-workers showed that 40% (8/20) of non-antral gastric adenocarcinomas aberrantly expressed GRP-R, and that in 6/8 positive tumors the receptor DNA was mutated [126]. At the protein level, GRP-R has also been detected on 20% (1/5) of gastric carcinoma cell lines, and characterized as a 37- to 44-kDa protein by covalent cross-linking [127]. In contrast in the human gastric carcinoma cell line SIIA an 80 kDa cell surface protein bound bombesin with both high (K_d=0.6 nM) and low (K_d=6.7 nM) affinities [128]. Tumors derived from the human gastric carcinoma cell lines MKN45 [129], Hs746T [130] and AGS [131] also expressed GRP-R when grown in nude mice.

5.2.5. Pancreatic cancer

GRP-R mRNA was detected in only 8% (2/26) [132], and GRP-R protein in only 17% (2/12) [133], of human pancreatic adenocarcinomas. A later study detected GRP-R in peri-tumoral small veins, but not in the tumor tissue, of 66% (19/29) of ductal adenocarcinomas [134]. Similarly no GRP binding sites were detected in the atypical acinar cell nodules induced in rat pancreas by treatment with azaserine [135], or in BOP-induced pancreatic preneoplastic lesions in hamsters [136], although an early report indicated that BOP-induced pancreatic tumors expressed GRP receptors [137]. In contrast cells isolated from an azaserine-induced rat pancreatic carcinoma were found to express high affinity GRP binding sites which corresponded to a 75-kDa glycoprotein [138].

Although the mRNAs encoding both GRP-R and NMB-R were detected by RT-PCR in the pancreatic carcinoma cell lines HPAF, CD18, CD11 and PANC-1 [107,139], high affinity bombesin binding sites were found on only two of the four lines [107]. A single class of high affinity bombesin binding sites was also detected on the rat pancreatic acinar carcinoma cell line AR4-2J, and characterized as an 80- to 85-kDa protein by covalent cross-linking [140]. The GRP antagonist BIM 26226 inhibited binding of [^{125}I]-Tyr^{15}-GRP to this cell line [141]. In contrast, the human pancreatic carcinoma cell line CFPAC-1 expressed both high and low affinity bombesin binding sites [142].

5.2.6. Colorectal cancer

Bombesin binds to many colon cancer cell lines via a single class of binding sites with an affinity in the 0.4 to 2.5 nM range [143]. An early report that the binding data for approximately half of the primary colon cancers expressing GRP-R was optimally fitted using a 2-site model [144] raised the possibility that the cell type affects the pharmacology of GRP-R or that GRP-R is mutated. Similarly, tumors generated by subdermal injection of MC-26 mouse CRC cells into male BALB/c mice displayed a high affinity (K_d=0.45 nM), as well as a 20-fold lower affinity binding site for radiolabelled bombesin [145]. Carroll and co-workers have reported that, although all colon cancer cell lines expressed similar amounts of mRNA for both GRP and GRP-R, functional receptors were detected on only 5/9 cell lines [111]. Sequencing revealed that the message for GRP-R contained between two and seven separate mutations which resulted in alteration of proline145 into a tyrosine, of valine317 into a glutamate, and insertion of a 32-nucleotide segment resulting in a frameshift distal to aspartate137. All mutations resulted in GRP receptors incapable of binding ligand [111]. The GRP-R gene was not mutated in non-malignant

epithelial cells, but 42 distinct mutations were identified in malignant cells [146]. The fact that the total number of mutations in the GRP-R gene was inversely correlated with the degree of tumor cell differentiation suggested that accumulation of mutations within the GRP-R gene ultimately resulted in the expression of a non-functional GRP-R, and hence blocked the maintenance of a differentiated state by GRP [146].

In contrast to GRP receptors in colorectal cancer cell lines, there are few reports of GRP receptors in colorectal tumors. A study by Radulovic and colleagues showed that 40% (6/15) of human colon cancer specimens had specific binding sites for ^{125}I-[Tyr4]-bombesin [144]. When colorectal cancer tissue and matched uninvolved mucosa from 21 patients was examined by radioligand displacement for the presence of GRP binding sites, 24% (5/21) of cancerous tissue, but no uninvolved mucosa, expressed the high-affinity GRP-R subtype [147]. RT-PCR detected widely variable GRP-R mRNA concentrations in 93% (27/29) of tumor specimens, but not in normal colonic epithelium [148]. Interestingly, concentrations of GRP-R mRNA were significantly higher in poorly/moderately differentiated tumors and in tumors with lymphatic vessel invasion [148]. A similar study by Chave and co-workers detected GRP-R mRNA in both tumor samples and matched normal mucosa, but concluded that expression was greater in the tumors than in normal mucosa [63]. Immunostaining of colon cancers using antibodies against GRP-R showed that 76% (38/50) were positive for GRP-R expression [109]. Patel and co-workers have shown that colorectal cancer cell lines (DLD-1, HT29 and LIM1215) express both GRP-R and BRS-3 receptor mRNA and protein [33].

5.2.7. Gastrointestinal carcinoid tumors

Investigation of the expression of GRP and GRP-R by immunohistochemistry in a panel of carcinoid tumors from the stomach, small intestine, appendix and colorectum revealed GRP-R in 85% (22/26) tumors. Co-expression of GRP and GRP-R was observed in 32% (6/19) of cases [114]. In contrast, in vitro receptor autoradiography detected NMB-R, but not GRP-R or BRS-3 binding sites, in 46% (11/24) of intestinal carcinoid tumors [116]. In pancreatic endocrine tumors GRP-R was detected by in vitro receptor autoradiography in 100% (5/5) of gastrinomas and in 100% (2/2) of VIPomas, but not in a somatostatinoma or a glucagonoma [149]. The presence of GRP-R, but not NMB-R or BRS-3, binding sites in 100% (5/5) of gastrinomas was confirmed independently [116].

In summary, a large proportion of human cancers have been shown to express mRNAs for GRP and/or its receptors by PCR. It should be emphasized again that detection of mRNA by PCR is not necessarily indicative of expression of the corresponding protein. However in many cases GRP and/or the GRP receptor have also been demonstrated at the protein level by radioimmunoassay or immunohistochemistry, and by radiolabelled ligand binding assay or autoradiography, respectively. Interestingly of the 14 studies that examined the expression of GRP-R in human tumors (Table 2), six detected GRP-R expression by PCR and eight by ligand binding assay or immunohistochemistry. Since approximately half of human tumors of various origins express GRP-R, the receptor presents an attractive target for cancer treatment.

6. Mitogenic role of GRP in cancer

It is now well established that GRP is a potent mitogen for various cancer cells in culture and in experimental models of carcinogenesis. GRP antagonists could therefore be an effective treatment for several malignancies. The sections below summarise the evidence for a mitogenic role of GRP in various cancers.

6.1. Lung cancer

GRP was mitogenic for SCLC cell lines, but not for either a squamous carcinoma or an adenocarcinoma lung cell line [150]. In a study in which a human SCLC cell line (NCI-H69) was subcutaneously implanted into the flanks of nude mice, bombesin treatment significantly increased tumor weight and DNA content [151]. A study using the synthetic octapeptides [D-Cpa^1-β-Leu^8-des-Met^9]litorin (BIM 26182) and [D-Phe^6, Leu^{13}-CH_2NH-Cpa^{14}]bombesin(6–14)NH_2 (BIM 26189) showed that these GRP antagonists are able to inhibit the in vitro and in vivo growth of GRP receptor-positive SCLC [152]. [Ψ13,14]bombesin analogues inhibited the increase in cytosolic Ca^{2+} and the growth of SCLC in vitro by approximately 70% and also inhibited the growth of SCLC xenografts in nude mice in vivo by approximately 50% [153]. Subcutaneous treatment of nude mice xenografted with H69 SCLC cells with the GRP antagonists RC-3095 and RC-3940-II for 5 weeks resulted in decreases in tumor volume of 50–70%. Tumor burden was also significantly decreased in both treated groups [154]. A cytotoxic analogue of bombesin (AN-215, a conjugate of 2-pyrrolinodoxorubicin and a bombesin antagonist (RC-3094)), inhibited growth of H69 SCLC xenografts in vitro and in vivo [155].

Some results are not consistent with the hypothesis that GRP-like peptides function as autocrine growth factors in SCLC. In a thorough study of 4 SCLC cell lines in vitro Layton and co-workers did not observe any stimulation of proliferation by bombesin at concentrations between 0.1 nM and 1 μM [156]. Furthermore, although GRP antagonists blocked proliferation, the concentrations required for 50% inhibition were approximately 10-fold higher than their IC_{50} values for binding to the GRP-R, and the inhibition was not reversed by exogenous bombesin.

6.2. Prostate cancer

The GRP antagonist RC-3095 inhibited the growth of Dunning R-3327-AT-1 prostate adenocarcinomas in rats. However, the remission produced by RC-3095 was of short duration, probably as a result of down-regulation of the GRP receptor [157]. Injection of the GRP antagonist RC-3095 subcutaneously for 4 weeks resulted in a 40% decrease in tumor volumes and weights in nude mice bearing xenografts of the androgen-independent human prostate cancer cell line PC-3

[158]. Similarly GRP antagonists inhibited the growth of the hormone-dependent human prostate cancer cell line PC-82 as xenografts in nude mice [159]. The GRP antagonists RC-3940-II and RC-3950-II also significantly reduced volume of tumors generated from the human prostate cancer cell line DU-145 in nude mice [160]. When tested in vitro, bombesin stimulated a concentration-dependent increase in cell growth of the DU-145 and PC-3 cell lines in the absence of any other exogenously added growth factors. Anti-bombesin antibody significantly inhibited both bombesin-stimulated and basal growth of these cell lines. Similarly, anti-bombesin antibody inhibited growth of DU-145 cells transplanted into mice. These results indicate that GRP may have an autocrine role in the growth of human prostate carcinoma [161]. Xiao and co-workers have demonstrated that bombesin induced proliferation in the DU-145 and PC-3 cell lines through activation of the transcription factor Elk-1 and the immediate early gene c-*fos* [162]. Bologna and co-workers have shown that growth of PC3 cells and of the epithelial cell line PMU 23 (derived from a primary culture of a stage III prostate carcinoma) was enhanced in a dose dependent manner by adding bombesin, and the increase was inhibited by antibodies against GRP [121]. Invasion and migration of PC-3, DU-145, TSU-pr1 and LNCaP prostate cancer cells was accelerated by GRP [163–165].

6.3. Breast cancer

Bombesin increased both cytosolic [Ca^{2+}] and cell growth in estrogen-dependent (MCF-7), estrogen-responsive (ZR-75-1) and estrogen-independent (MDA-MB-231) human breast cancer cell lines. These effects were blocked by a GRP antagonist [166]. In addition, when the MDA-MB-231 cell line was xenografted in nude female mice the GRP antagonists RC-3940-II and RC-3095 significantly decreased the final tumor volume and weight [167]. Treatment of nude mice bearing xenografts of MCF-7 MIII human breast cancer cells with the GRP antagonists RC-3950-II and RC-3095 significantly decreased the final tumor volume and weight [168].

6.4. Gastric cancer

Bombesin significantly stimulated the proliferation of the human gastric carcinoma cell lines SIIA [169] and HS746T [130] in vitro. Xenograft growth of the human gastric carcinoma cell lines MKN45 [170] and HS746T [130] was significantly inhibited by treatment with the GRP antagonist RC-3095. Bombesin significantly increased the incidence of peritoneal metastases from gastric cancers induced in rats by treatment with a chemical carcinogen [171]. However, the survival of patients with gastric carcinoma that expressed functional GRP-R was not significantly different from patients with GRP-R-negative tumors [109].

6.5. Pancreatic cancer

Investigation of the effects of bombesin in two rodent models of pancreatic carcinogenesis has produced conflicting results. Bombesin promoted the development of atypical acinar cell nodules in rat pancreas, and increased the number of pancreatic tumors after treatment with azaserine [172]. In contrast, bombesin treatment reduced the numbers of BOP-induced preneoplastic pancreatic ductular lesions in hamsters [172]. Surprisingly, the GRP antagonist RC-3095 also reduced the number of pancreatic tumors in this model [137,173], and a greater reduction was observed when GRP and RC-3095 were administered together [173]. Later studies did not detect bombesin binding sites in either model [135,136].

Several groups have reported stimulation of growth of established pancreatic carcinomas by GRP. Xenograft growth of an azaserine-induced pancreatic carcinoma in Lewis rats or in vitro was stimulated by administration of GRP [174], and the stimulation was reversed by the GRP antagonist BIM 26226 [175]. The tumor tissue expressed GRP-R, and the dose–response curve for stimulation of tumor cell growth in primary culture closely matched the receptor binding curve [174]. Bombesin also stimulated the xenograft growth of a human pancreatic gastrinoma [176]. The xenografts expressed more NMB-R mRNA than GRP-R mRNA, and an antagonist study suggested that occupation of NMB-R was responsible for the observed growth stimulation [177]. The presence of an autocrine loop involving GRP and the GRP-R was suggested by the observation that the GRP antagonists RC-3095 and RC-3940-II inhibited xenograft growth of the human pancreatic adenocarcinoma cell lines CFPAC-1 [142] and SW-1990 [178].

Treatment of the human pancreatic cancer cell lines Capan with GRP [179], and CFPAC-1 [142], MIA PaCa-2, PANC-1 [180], and LPC5 [181] with bombesin, significantly increased proliferation. Treatment of Capan cells with an anti-GRP-R monoclonal antibody [179], or of Capan or CFPAC-1 cells [142] with a GRP antagonist, blocked GRP-stimulated proliferation. Bombesin also significantly stimulated the proliferation of well-differentiated (HPAF, CD11) and poorly differentiated (CD18, PANC-1) human ductal pancreatic cancer cell lines, and this trophic effect was inhibited by the GRP antagonist RC-3095 [107,139].

6.6. Colorectal cancer

Bombesin significantly increased the number of colorectal cancers induced in rats by treatment with azoxymethane or 1,2-dimethylhydrazine, without affecting the size or histological type of the tumors [82,83]. The incidence of peritoneal metastases induced by treatment with azoxymethane was also increased by bombesin treatment [82,182].

GRP is a mitogen for several colorectal cancer cell lines in vitro and GRP antagonists block the proliferative effect of GRP [112,143]. A significant dose-dependent stimulation by bombesin (0.5–50 nM) of growth of the mouse CRC cell line MC-26 was demonstrated using two independent proliferation assays [145,183]. In cell culture after partial serum deprivation (1% fetal bovine serum) for 48 h the number of HT-29 cells was increased after stimulation with bombesin or GRP for 24 h [112]. Two different GRP antagonists ([D-Phe^6]bombesin(6–13)methyl ester and BIM-23127) inhibited bombesin/GRP

stimulated growth of HT-29 cells. Surprisingly these antagonists stimulated proliferation when cells were treated with antagonist only [112]. Bombesin or GRP significantly increased [^{3}H]-thymidine uptake by Isreco1 and DLD-1 colon cancer cells and these effects were blocked by GRP antagonists [33,143]. In contrast, bombesin did not stimulate proliferation of the human CRC cell lines COLO 320, LoVo and HCT116, despite mobilizing intracellular Ca^{2+} [184].

GRP is also mitogenic for colorectal cancer cell lines in vivo. Bombesin stimulated the growth of the mouse CRC cell line MC26 as xenografts in nude mice, and reduced the survival time of the xenografted mice [185]. Treatment of nude mice bearing xenografts of the HT-29 human colon cancer cell line with the GRP antagonist RC-3095 for 4 weeks, by subcutaneous injections (20 μg/day) or by continuous infusion using Alzet osmotic minipumps, resulted in significant decreases in tumor volume and weight compared to control [186,187].

A second possible mechanism of growth stimulation may be constitutive activation of the GRP-R. Benya's group has reported that transfection of the non-malignant human colon epithelial cell line NCM460 with a plasmid encoding the human GRP-R resulted in expression of a constitutively active receptor [188]. The transfected cells proliferated independently of serum or other growth factors, and their growth was unaffected by addition of GRP antagonists.

7. Morphogenic role of GRP

Morphogens are proteins normally involved in organogenesis that when re-expressed by various cancers act to maintain the malignancy in a better differentiated state. Studies have shown that GRP-R expression is seen in well differentiated but not in poorly differentiated SCLC and prostate cancers (reviewed by [189]). When GRP/GRP-R expression was studied in 50 randomly selected human colon cancers GRP/GRP-R co-expression was only observed in well-differentiated tumors while poorly differentiated tumor regions never co-expressed these 2 proteins [109]. The same study also found that GRP/GRP-R co-expression did not impair patients' survival. In contrast, a French group has reported that concentrations of GRP-R mRNA detected by RT-PCR were significantly higher in poorly/moderately differentiated colorectal adenocarcinomas and in tumors with lymphatic vessel invasion [148]. Another study comparing cell proliferation in azoxymethane-induced colorectal tumors in wild type mice to GRPR−/− mice found that although the tumor cells with GRP-R proliferated 3-fold more rapidly than cells without GRP-R, the tumors from both types of mice were of the same size, largely because the increased proliferation rate in wild type mice was precisely balanced by an increase in tumor cell apoptosis [190]. These authors then proposed that although GRP is a mitogen, a more important role especially in colorectal cancer is that of a morphogen [189].

GRP appears to alter cell shape as well as cellular adhesion by altering the actin cytoskeleton through activation of focal adhesion kinase (FAK). Studies have shown that the amount of total FAK and of FAK phosphorylated at tyrosine397 and tyrosine407 tightly correlates with differentiation and with the amount of GRP/GRP-R co-expression [191,192]. Alterations in the cytoskeleton may also be a prelude to cell migration. since GRP has been shown to stimulate invasion and migration of the colon carcinoma cell line Isreco-1 [143] through a Rho-dependent pathway [193]. The strong association of GRP/GRP-R co-expression with tumor differentiation raises the possibility that GRP/GRP-R expression in colorectal cancer is critical to regulating tumor cell morphology, cell–cell and cell–matrix attachment, and cell migration [189].

8. Biological activity of non-amidated GRPs

Recent data has shown that prohormones [194–196] and prohormone-derived peptides [197–200] which were originally thought to be inactive may in fact be active via novel receptors. Thus although the amidated forms of peptide hormones were thought for many years to be the only biologically active species [201], in the last decade several glycine-extended hormone precursors such as gastrin and secretin have been shown to be active [202,203]. In the case of GRP, processing of the prohormone gives rise to both amidated and glycine-extended forms, including GRP_{1-27}gly and GRP_{18-27}gly. Studies have shown that the glycine-extended form of bombesin is biologically active in Swiss 3T3 cells, in pancreatic acini and in the SCLC cell line NCI-H345 [204,205]. Recently, Patel and co-workers have shown that the glycine-extended form of GRP (GRPgly) stimulates proliferation and migration in colorectal cell lines [33].

Non-amidated, C-terminally extended forms of GRP have been detected in mammalian tissues and tumors. The reproductive tracts of the human and sheep produce large amounts of higher molecular weight forms of GRP [27,28], but their bioactivity has not yet been tested. An extensive study by Hamid and co-workers evaluated 505 (361 neuroendocrine) surgically resected pulmonary tumors for the presence of immunoreactive GRP and the C-terminal peptide of proGRP [206]. Strong immunostaining was observed with an antiserum to the C-terminal peptide in 70% (175/250) of the small cell carcinomas, and in 63% (31/49) of atypical (aggressive) carcinoids, but in only 16% (10/62) of benign carcinoids. In contrast, immunostaining for amidated GRP was focal and only moderately strong, and the relative proportion of positive cases was evenly distributed amongst the neuroendocrine tumors as follows: 35% (22/62) of carcinoids, 22% (11/49) of atypical carcinoids and 25% (62/250) of small cell carcinoma [206]. None of the squamous, adeno-, or large cell undifferentiated carcinomas was immunoreactive for GRP or the C-terminal peptide. Moreover, patients with tumors showing immunoreactivity to the C-terminal peptide of proGRP had a significantly shorter survival time (185 days) than those without immunoreactive peptide (1128 days) [206]. This observation led to further study of the expression and secretion of proGRP. Patients with SCLC, prostate cancer and medullary thyroid cancer have elevated circulating concentrations of proGRP as detected using antiserum raised against $proGRP_{31-98}$ [103,207,208]. This increase has been corroborated in patients

with SCLC using an antisera raised against $proGRP_{42-53}$ [96]. Hence proGRP may be a serum marker for a variety of cancers. Although large amounts of proGRP are secreted, the circulating forms have not yet been characterized.

The biological activity of peptides derived from the C-terminus of proGRP has not been extensively studied. Walsh and co-workers showed that synthetic peptides corresponding to the non-conserved regions within the proGRP sequence including $proGRP_{31-52}$ (Forms I, II and III), $proGRP_{103-125}$ (Form I) and $proGRP_{99-115}$ (Form III) were unable to stimulate acid secretion or gastrin release in dogs [209]. In contrast Reeve and co-workers identified a specific high affinity-binding site for $proGRP_{99-115}$ (Form III) on the SCLC cell line NCI-N592, and the binding of this peptide was not inhibited by high concentration of amidated GRP [210].

9. Prospects for therapy

From the above literature, it is apparent that GRP receptors are over-expressed in small cell lung, prostate, gastric, breast, pancreatic and colorectal cancers. Phase I clinical trials using an antibody against GRP have been conducted in patients with lung cancer [211]. Further investigation of GRP antagonists in the treatment of human cancer could provide substantial benefit to cancer patients. Zhou and co-authors have reviewed in detail some of the strategies for targeting GRP receptors for therapy [4]. The recent observations that GRP stimulates angiogenesis and that a GRP antagonist blocks tumor growth and angiogenesis present additional new therapeutic options [52].

Several antibody-based strategies have been investigated (for review, see [4]). Early work from Cuttitta and co-workers showed that a mouse monoclonal antibody against GRP was able to block proliferation of SCLC cell lines [1]. The efficacy of the same antibody has also been investigated in phase I and II trials with SCLC patients [211,212]. Of the thirteen patients observed in the phase II trial, stable disease was achieved in four, and the disease was eradicated in one [212]. In an alternative approach, a GRP-R agonist or antagonist was coupled to a monoclonal antibody against the Fc receptor, in order to redirect immune effector cells to tumor cells that expressed the GRP-R. In both cases lysis of SCLC tumor cells in vitro was enhanced [213,214].

Cytotoxic peptide conjugates, also known as magic bullets [215] or prodrugs [216] are promising alternatives to peptide receptor antagonists. In the case of peptide hormone prodrugs, the cytotoxic agent is coupled to a peptide hormone through a chemical linkage. The conjugate retains high affinity for the peptide hormone receptor but the cytotoxic drug is released after receptor-specific internalization and degradation [217]. Since receptors of the GRP-R family are over-expressed in a number of tumors, GRP-R ligands are receiving considerable attention as vehicles to deliver cytotoxic agents (reviewed by [215]). Cytotoxic bombesin analogues have been used to inhibit the growth of cancers and cell lines of different origins including ovarian [218], gastric [131], prostate [219], small-cell lung [155], and pancreatic carcinoma [220], and glioblastoma [221]. Recent developments have been the synthesis of doxorubicin–bombesin conjugates [131,220] and camptothecin–bombesin conjugates for targeted killing of cancer cells expressing GRP-R [222]. Recently, a small non-peptide GRP antagonist has been developed and shown to be effective in inhibiting angiogenesis in vivo as well as in vitro [52]. With the help of computer-assisted drug design it should be possible to develop additional small GRP antagonists with high affinity and specificity for the GRP receptor.

Tumor imaging and detection is the other area which has exploited the fact that GRP receptors are overexpressed in a number of tumors [223]. GRP-like peptides labelled with β- and/or γ-emitting radionuclides are currently being investigated for their ability to bind to cell-surface GRP receptors, and hence for their potential utility for detection and/or therapy for tumors (reviewed by [224,225]). Currently, the radionuclides used include Gallium-67 [226], Gallium-68 [226], Yttrium-90 [227], Technetium-99m [228,229], Indium-111 [227], Lutetium-177 [227], and Rhenium-188 [230]. For example Technetium-99m–Leu^{13}–bombesin has been used for detection of colorectal cancers by whole body scanning [231].

10. Summary and future directions

From the foregoing discussion, it is obvious that amidated GRP is a mitogen for many lung cancers. However, the situation with gastrointestinal and prostate cancer is less clear with active debate over the presence and nature of GRP, the biological activity of GRP, the frequency and subtype of GRP receptors, and the mechanisms of their action. With the growing number of studies reporting biological activity of non-amidated forms of peptide hormones, another major issue that needs to be clarified is whether glycine-extended GRP_{18-28} (GRPgly) or other non-amidated fragments derived from the C-terminus of proGRP have any biological activity especially in cell lines derived from gastrointestinal cancers. Recent reports have extended the range of tumor type in which the GRP family of peptides and receptors may play a role to include ovary [218,232], brain [233,234], kidney [235,236], skin [237,238] thyroid [239,240], uterus [241] and neuroblastoma [234,242]. Further investigation of the actions of GRP and GRP-R in cancer development and progression is clearly warranted.

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